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Grantee Institution: University of Massachusetts Medical School
Progress, Status and Management Report
Progress Report

Period Covered by the Report
[April 1st 2014] through [July 31st 2014]

Date of Report: August 14, 2014

Project Title: Evolutionary profiling of the transcriptional and post-transcriptional wiring of the circadian clock in normal and perturbed conditions
Contract Number: DARPA D13AP00074
Total Dollar Value: $157,942
Program Manager: COL Christian Macedonia, Defense Advanced Research Projects Agency

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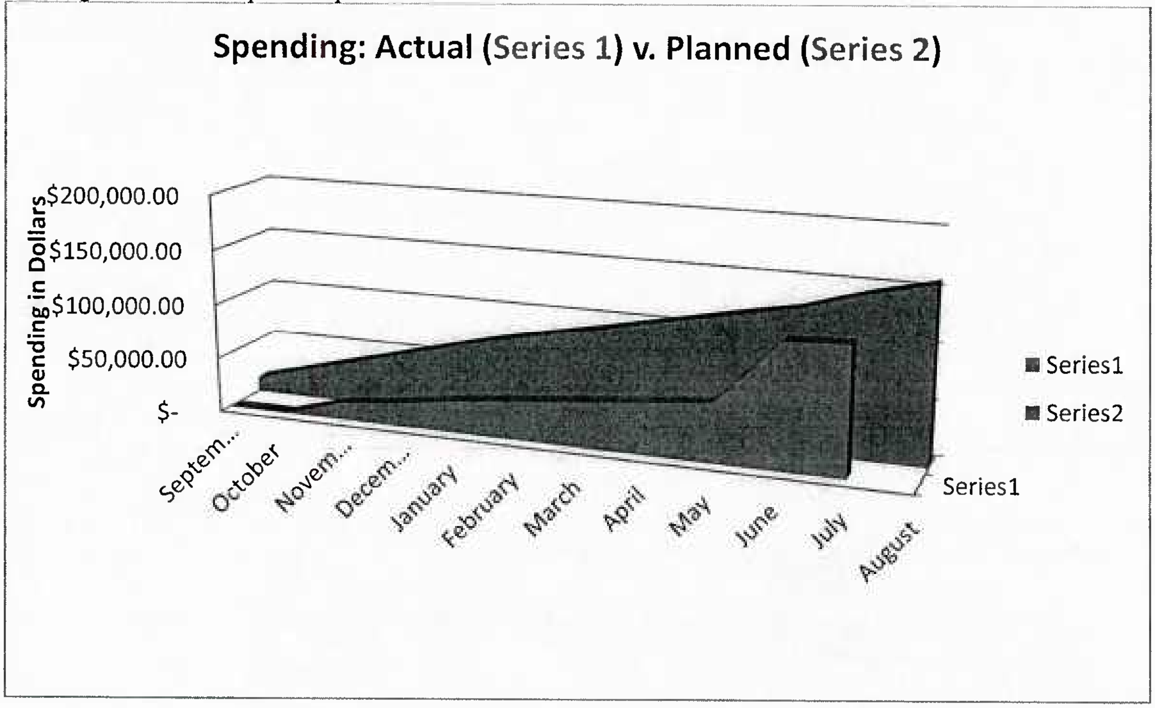
Technical Information – Financial Management

1. **Technical Progress / Monthly Expenditure Report (Please provide cumulative spending graph).**

Figure 1. Example Graph



Please provide Phase 1 schedule of tasks and events for this quarter, with financial expenditures broken down by task. Please justify any large discrepancies between planned and actual spending.

Summary of major direct costs:
Salaries (Computational group) - $30,000
Benefits (Computational group) - $9,000
Supplies – $5,000
Sequencing & Salaries (Subcontract, Israel) – $26,000

Actual Cost versus Planned Costs

	Inception to Date ($)	Phase 1 Cost ($)
Plan	$144,460.94	157,000
Actual	$114,465.99	157,000
Difference	-30,000	0

Justification of under-spending: There was some delay in setting up the subcontract at Israel. Expenditures by the subcontract have not yet been fully processed. We had planned to complete the comparative sequencing dataset by June. Although the first libraries failed in late May. Our collaborator redid sample collection, RNA-Extraction and library construction. Sequencing data for the new libraries arrived on August 10. We are currently analyzing these datasets and preliminary results suggest that all libraries are usable. We also expect a charge from our colleague at the Hebrew University for the budgeted amount now that their libraries are producing good quality data.

Until this point we had mapped and optimized the timing and temperatures to target for analysis. Here I append our previous results:

Results

Behavioral analysis of fly species. As we described on our proposal we carried out behavioral analysis of the different fly species and their response to temperature changes. Towards this, we tested the circadian locomotor activity of eight different species of drosophila from different parts of the world, that have adapted to different environmental conditions in three different temperatures and compared the results to the well-studied *Drosophila melanogaster*. We kept the flies in 12 hours light 12 hours dark cycles and monitored the flies locomotor activity during seven days at 18C°, 25C° and 29C°. In addition we transferred part of the flies from 25C° to 18C° and from 25C° to 29C° in the fourth day of the experiment. We have tested the influence of the temperature transfer in two time points; flies were transferred either three hours after light on (ZT3) or three hours after light off (ZT15).

Overall we observe some drastic change in behavior with temperature. For example D. *pseudobscura* and D. *persimilis* show a switch from nocturnal to almost diurnal behavior when grown at 29C° vs 18C°. D. melanogaster immediately switches to nocturnal behavior when transferred from 25C° to 29C°. This provides us with the optimal fly systems in which to study how the circadian cycle adapts to environmental stress. More specifically:

As expected, *Drosophila melanogaster* showed two peaks of activity: one in the morning and one in the evening. The evening peak timing was more diurnal at 18C°compare to 25C°, while in 29C° it shifted further to the night. As reaction to the temperature transferred from 25C° to 18C° or to 29C°, *Drosophila melanogaster* flies immediately changed their behavior patterns to patterns of 18C° or 29C° respectively. This respond accrued in both ZT3 and ZT15 temperature transfer.

Drosophila ananassae showed morning peak and evening peak and almost no activity at night. The morning peak was wider at 18C° in compare to 25C° and 29C°. The pattern of activity was similar at 25C° and 29C°. After temperature transition from 25C° to 18C° the flies showed lower levels of locomotor activity comparing to flies that were constantly at 18C°. The activity pattern change in respond to transition to 18C°, accrued immediately in both ZT3 and ZT 15. Temperature transfer from 25C° to 29C° did not change flies behavior.

Drosophila virilis showed wide evening peak at18C°and no defined peak of activity at 25C° and 29C°. The pattern of activity was similar at 25C° and 29C°. After temperature transition from 25C° to 18C° the flies showed lower levels of locomotor activity comparing to flies that were constantly at 18C°. The activity pattern change in respond to transition to 18C°, accrued immediately in both ZT3 and ZT15. Temperature transfer from 25C° to 29C° did not change flies behavior.

Drosophila willistoni showed wide evening peak and narrow morning peak at 25C°and 29C°. However, at 18C° it did not show any defined peak of activity. The pattern of activity was similar at 25C° and 29C°. Temperature transfer from 25C° to 29C° did not change flies behavior. The activity pattern change in respond to transition to 18C°, accrued immediately in both time points. However, flies that were transferred to 18C° showed evening peak in the three days tested after temperature transition.

Drosophila simulans showed evening peak. At 18C° the evening peak is wider and shifted to the day time comparing to 25C°and 29C°. The evening peak at 29C° has less activity during the light phase compering to the evening peak at 25C°. Temperature transfers from 25C° to 29C° immediately reduced the activity during the light phase in both temperature transitions time points. The activity pattern change in respond to transition to 18C°, accrued immediately in both time points. However, flies that were transferred to 18C° showed smaller evening peak than the flies that were kept constantly at 18C° in the three days tested after temperature transition.

Drosophila mojavensis shows in general low activity levels. The flies showed evening peak in 18C° 25C°and 29C°. After transition to 18C°, in both tested time points, the evening peak disappeared, the peak is gradually reappear in the following days. Transition to 29C° did not cause string behavior change in both temperature transition time points.

Drosophila yakuba showed morning and evening peaks in the 3 tested temperatures. The activity levels during the light phase were smaller at 29C°. The respond to temperature transition was very mild.

Drosophila persimilis showed morning and evening peaks. The activity levels were smaller at 29C°. At 18C° the evening peak was wider and shifted to the day time comparing to 25C°and 29C°. In respond to transition to 29C° at two time points the flies increased night activity in the following days, while transition to 18C° led to day activity increase.

Drosophila pseudoobscura showed evening peak, which is mainly nocturnal in 25C° and 29C° and mainly diurnal at 18C°. After several days at 29C° activity levels were largely decreased. After transition to 29C° flies locomotor activity is gradually decrease. After transition to 18C° flies showed more diurnal activity and the activity levels were lower than the activity levels of flies that were constantly at 18C°.